Targeting CDK12 for Cancer Therapy: Function, Mechanism, and Drug Discovery

Hui Liu[1], Kangdong Liu[1,2], and Zigang Dong[1,2]

ABSTRACT

Cyclin-dependent kinase 12 (CDK12) is a member of the CDK family of proteins (CDK) and is critical for cancer development. Years of study into CDK12 have generated much information regarding the intricacy of its function and mechanism as well as inhibitors against it for oncological research. However, there remains a lack of understanding regarding the role of CDK12 in carcinogenesis and cancer prevention. An exhaustive comprehension of CDK12 will highly stimulate the development of new strategies for treating and preventing cancer. Here, we review the literature of CDK12, with a focus on its function, its role in signaling, and how to use it as a target for discovery of novel drugs for cancer prevention and therapy.

Introduction

Cyclin-dependent kinase 12 (CDK12) is one of the 20 members of cyclin-dependent kinases (CDK). Each of CDKs binds with cyclin play important roles in the control of cell cycle and cell division and regulates transcription in response to various cellular processes. CDKs can be divided into two categories: one is cell cycle–related CDKs, including CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK14, CDK15, CDK16, CDK17, and CDK18; and the other is transcription-related CDKs (**Fig. 1A**). There are extensive studies regarding CDK4, CDK6, CDK7, and CDK9 (1, 2). However, functions of several other CDKs remain largely unknown, such as CDK15, CDK16, CDK17, CDK18, CDK19, and CDK20. The comparisons of key features (subcellular location, molecular weight, cyclin partner, chromosomal location, molecular function, and key biological process) of representative CDKs are summarized in **Table 1**. Among the CDK family members, CDK12 and CDK13 possess the highest sequence homology with largest molecular weight. CDK13 has some similar biological functions with CDK12 because of the similar sequences; however, studies on CDK13 are not abundant yet. Among all CDKs, only CDK12 locates on the chromosome 17q12, which always contains oncogenic features and genetic alterations in various tumors (3). In this review, we mainly focus on CDK12.

Initial studies of CDK12 used the Ctk1 metazoan counterpart in *Drosophila* (4). CDK12 is also named as Cdc2-related kinase (CrkRS) and cell division cycle 2-related protein kinase 7 (CRK7; refs. 5, 6). CDK12 encompass two proline-rich motifs that are critical for protein–protein interactions, a carboxy-terminal kinase domain (KD) and an arginine/serine (RS) domain (**Fig. 1A**), which is commonly found in splicing factors of the RS-rich family (5). CDK12 mainly localizes in the nucleus and cytoplasm, which is important for embryonic development and the maintenance of genomic stability. Knockout of CDK12 ($CDK12^{-/-}$) leads to embryonic lethality (7). Other CDKs knockout mouse models phenotypes were summarized in **Table 1**. And CDK12 knockdown in cortical neurons decreases the length of averaged axonal by regulating CDK5 expression (8).

The cyclin partners of CDK12 initially identified were cyclin L1 and L2 (9). Cyclin K was later proved to be the credible CDK12-interacting cyclin, which is critical for CDK12 kinase activity (10–12). CDK12/Cyclin K complex plays important roles in gene expression regulation by phosphorylating RNA polymerase II (RNA Pol II; ref. 12). CDK12 can also regulate RNA splicing through interaction with RNA processing factors (13). Furthermore, CDK12 couples mRNA 3′ end processing via phosphorylation of RNA Pol II (14). Besides, CDK12 takes a significant role in regulating intronic polyadenylation and translation (15–17). CDK12 alterations including mutations, amplifications, fusions, and deletions were found in different human cancers (18–21). CDK12 is crucial in tumor progression by regulating c-MYC expression, WNT/β-catenin signaling, ErbB–PI3K–AKT signaling, MAPK signaling as well as noncanonical NF-κB pathway, and DNA damage response (DDR) signaling (22–26). It is recognized that CDK12 can act as both a biomarker and a therapeutic target in different cancer types (2, 25, 27–29). Inhibitors of CDK12 were developed and a number of studies have proved that a CDK12 inhibitor is highly effective for targeted therapy and combination therapy with anticancer drugs as well as *de novo* therapeutic resistance in several cancers (30–34). Herein, we summarize the literatures on CDK12 from the function, mechanism, and inhibitors, highlighting that it is an effective and promising therapeutic target in different types of human cancers.

Structure-Based Functional Role of CDK12 on DNA Replication, Transcription, Splicing, and DNA Damage Repair

Phosphorylation of cyclin E1 at Ser366 mediated by CDK12/cyclin K restrains the interaction of CDK2 with cyclin E1 in early G_1 phase, which suggests a novel role of CDK12 in regulating cross-talk between replication of DNA and transcription (Supplementary Fig. S1A; ref. 35). As a kinase, CDK12 phosphorylates the RAN Pol II of carboxy-terminal domain (CTD) at Ser2 and Ser5 *in vitro* and phosphorylates at Ser7 preferentially when it is prephosphorylated (14, 36). In addition, other

[1]Department of Pathophysiology, School of Basic Medical Sciences, The Academy of Medical Science, College of Medical, Zhengzhou University, Zhengzhou, Henan, China. [2]China-US (Henan) Hormel Cancer Institute, Jinshui District, Zhengzhou, Henan, China.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Author: Zigang Dong, School of Basic Medical Sciences, The Academy of Medical Science, College of Medical, Zhengzhou University, Zhengzhou, Henan 450001, China. Phone: 371-6665-8803; Fax: 507-437-9606; E-mail: dongzg@zzu.edu.cn

Cancer Res 2021;81:18–26

doi: 10.1158/0008-5472.CAN-20-2245

non-CTD substrates and CDK12-binding partners have characterized by phosphor–proteomics and affinity–purification mass spectrometry. These non-CTD substrates are categorized as multiple protein kinases and RNA-splicing factors, with examples such as CDK5, mRNA 5′ cap-binding repressor, 4E-BP1, noncanonical NF-κB, p21-activated kinase 2 (PAK2; refs. 8, 17, 24, 26). However, the exact consequences of the phosphorylation and binding of these non-CTD CDK12 partners remain incomplete and are not well studied.

The RS motifs of CDK12 may play a role in pre-mRNA processing, CDK12 colocalizes with SC35 (a component of spliceosome, SRSF2 or SFRS2) to nuclear speckles and spliceosome, and thus is believed to be important in RNA splicing (5). CDK12-related proteins may be involved in RNA processing factors, RNA splicing machinery, as well as ALE (alternative last exon) splicing of genes containing large number exons and long transcripts (10, 13). CDK12 couples transcription and mRNA 3′ end processing by phosphorylating Ser2-RNA Pol II and interacting with the process of polyadenylation and termination machinery, recruiting the breakage and polyadenylation factor CstF77, leading to efficient 3′ end formation (Supplementary Fig. S1B; refs. 37, 38). CDK12 is required for MYC first rank pre-mRNA processing, with reduced levels of polyadenylated MYC RNA by gene depletion, indicating that CDK12 is critical for regulating intronic polyadenylation (Supplementary Fig. S1C; ref. 38). Despite the limited evidence shows that CDK12 is involved in splicing, the precise mechanisms are not fully understood. It remains for further study on mechanism to elucidate how gene selectivity is accomplished and what other partners involved in this process.

CDK12 depletion does not change transcription globally, but only alters a subset of genes associated with DDR. The most altered genes are those with large number of exons, which includes BRCA1, ATR, FANCI, and FANCD2 (11, 14). Loss of CDK12 leads to reduced transcription of BRCA1 and increased sensitivity to DNA-damaging agents. More importantly, mutations of CDK12 kinase domain lead to impairment of DNA double-strand breaks (DSB) through homologous recombination (HR; ref. 11). CDK12 is required for the expression of oxidative stress response-related genes and function of stress-activated Nrf2 transcription factor (39). In summary, CDK12 regulates the particular subclass of genes that participate in cellular responses to DNA damage, stress, and heat shock (11, 39, 40). However, molecular mechanisms for this specificity remain not entirely understood.

Mutations of CDK12 in Tumor

CDK12 has the highest alteration frequency among all CDKs with large percentage of mutations, amplifications, fusions, and multiple alterations (**Fig. 1B**). Hundreds of mutations spread over the whole protein sequence in different domains (**Fig. 1B**). Genomic alterations of CDK12 have identified in different human cancer types, including esophageal cancer, bladder cancer, colon cancer, prostate cancer, breast cancer, lung cancer, ovarian cancer, and other cancers (**Fig. 1B**). The high mutation frequency tissues belong to ovary, adrenal gland, stomach, breast, and urinary tract among different human tissues (**Fig. 1B**), but there is no more study of CDK12 mutations in other tissues besides ovary, prostate, breast, and lung.

CDK12 Mutations Lead to Genomic Instability and Defects of DNA Damage Repair

CDK12 mutation frequency ranks the top 3 in patients with advanced non–small cell lung cancer (NSCLC) with bone metastasis. A nonfunctional novel CDK12 site mutation (G879V) in NSCLC can cause genomic instability and enhance sensitivity to chemotherapy (19, 41, 42). It was reported that a 61% mutation rate of CDK12 in 107 Chinese patients with HER2-positive breast cancer (43). CDK12 locates proximal to HER2 at approximately 200 kb; they are frequently coamplified in breast tumors, which accounts for around 90% of HER2-positive breast cancer (13, 25, 44, 45). The homozygous point mutations in the kinase domain of CDK12 are the main mutations in high-grade serous ovarian carcinoma (HGSOC), which abolish the catalytic activity of CDK12, cause multiple DNA repair pathways defects, leading to decreased HR and genomic instability (46, 47).

CDK12 Mutation and Other Forms of Alteration May Be a Potential Biomarker in Cancer

The well-studied mutation of CDK12 belongs to tandem duplications (TD), mainly in ovarian and prostate cancer (20). TD is a structural rearrangement that generates physically contiguous, head-to-tail duplications of a segment of DNA. The phenotypes of TD was uncovered in tumors show no obvious single coding or noncoding location, producing hundreds of small copy number gains, suggesting a systemic etiology rooted in a defect in DNA repair or replication, specifically, interfering with heterogeneous combinations of tumor suppressors and chromatin topological associated domains, while simultaneously replicating oncogenes and super enhancers (48–50). The number of focal tandem duplications (FTD) is significantly increased in CDK12 loss of function cases compared with CDK12 wild-type cases in ovarian, breast, gastric/esophageal, and endometrial cancer (20). The rate of CDK12 loss is around 3% to 7% in patients with metastatic prostate cancer, with a genomic instability signature, and patients with CDK12 mutation exhibited fast metastasis from original cancer and progression of castration-resistant disease compared with other genomic subtypes. It also showed fast progression on first-line ARPI (androgen receptor pathway inhibitor) treatment of metastatic castration-resistant disease (51–56). There are evidences suggesting that inactivating CDK12 alterations in prostate cancer correlates with poor outcomes to hormonal, taxane, and PARP inhibitors, while promoting sensitivity to immunotherapy (20, 57, 58). It is reported that CDK12 mutation was correlated with poor disease-free survival in Chinese patients with prostate cancer (59). The retrospective analysis of CDK12 polymorphisms in patients with late-stage epithelial ovarian cancer revealed that the G/G genotype of CDK12 polymorphism (rs1054488) predicted worse overall survival and progression-free survival than the genotype A/A-A/G (60). Several clinical trials evaluating CDK12 mutational status as a biomarker in various cancer types have been conducted (Supplementary Table S1; ref. 2). All these findings indicate that CDK12 alterations may be a potential biomarker for response to immune checkpoint blockade in cancer.

CDK12 Participates in DDR and Checkpoint Signaling

Cell-cycle processes consistent with DDR mechanisms ensure genomic stability. Cell division or exogenous DNA-damaging agents induce DSBs of DNA that can cause genomic instability. Aborted DSBs repair may cause chromosome breakage or rearrangement. These two kinds of chromosomal damages may be repaired by HR and NHEJ (nonhomologous end joining)

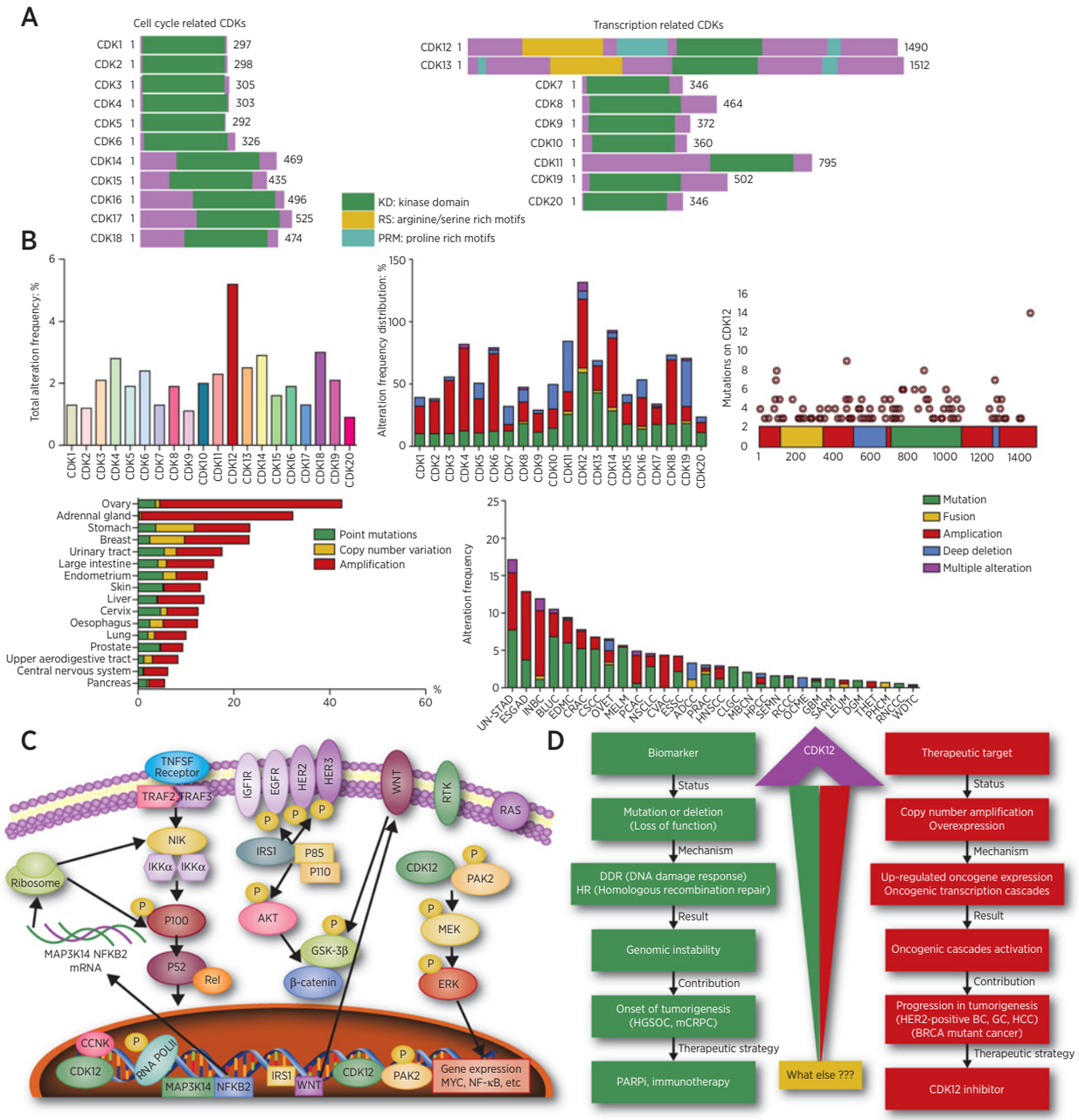

Figure 1.

Structure, mutation, signal transduction, and overall review of CDK12. **A,** The schematic structure of all CDKs. **B,** Total alteration frequency/alteration frequency distribution of all CDKs and mutations (more than 3) of CDK12 on protein sequence from The Cancer Genome Atlas and COSMIC (top). Mutation rate of CDK12 in different human tissues (top 16) and different cancers according to The Cancer Genome Atlas database (bottom). UN-STAD, undifferentiated stomach adenocarcinoma; ESGAD, esophagogastric adenocarcinoma; INBC, invasive breast carcinoma; BLUC, bladder urothelial carcinoma; EDMC, endometrial carcinoma; CRAC, colorectal adenocarcinoma; CSCC, cervical squamous cell carcinoma; OVET, ovarian epithelial tumor; MELM, melanoma; PCAC, pancreatic adenocarcinoma; CVAV, cervical adenocarcinoma; ESSC, esophageal squamous cell carcinoma; ADCC, adrenocortical carcinoma; PRAC, prostate adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; CLGC, cholangiocarcinoma; MBCN, mature B-cell neoplasms; HPCC, hepatocellular carcinoma; SEMN, seminoma; RCCC, renal clear cell carcinoma; OCME, ocular melanoma; GBM, glioblastoma; SARM, sarcoma; LEUM, leukemia; DGM, diffuse glioma; THET, thymic epithelial tumor; PHCM, pheochromocytoma; RNCCC, renal non–clear cell carcinoma; WDTC, well-differentiated thyroid cancer. **C,** CDK12 promotes the ligand-induced increase in the abundances of MAP3K14 and NFKB2 mRNAs, enhancing activation of the noncanonical NF-kB pathway. CDK12 promotes tumor initiation, invasion, and metastasis, as well as maintains cancer stem cell self-renewal via activating ErbB–PI3K–AKT or WNT/β-catenin signaling in breast cancer. CDK12 binds with and phosphorylates PAK2 to activate MAPK signaling pathway. **D,** CDK12 acts as a biomarker and therapeutic target for cancers. When there is CDK12 loss of function in cancer, it causes genomic instability and enhances the therapeutic sensitivity to PARP inhibitors and immunotherapy. (*Continued on the following page.*)

typically (61–65). CDK12 protein absence in breast cancer cells may reduce the expression of many DDR-related proteins including ATR, γH2AX, PARP1, DNA-PK, and Ku70/Ku80. Lacking of CDK12 will result in defects in gene length-dependent elongation, inducing PCPA (premature cleavage and polyadenylation) and missing of long genes expression (>45 kb). CDK12 phosphorylates pre-mRNA processing factors directly, including those genes regulating PCPA. DDR genes may susceptible to CDK12 inhibition (15, 16, 25). CDK12 can work with mTORC1 and control a specialized translation network that is critical to the stability of mitotic chromosomes (17). Cell-cycle checkpoints are used in situations that are detrimental to complete cell division, such as when DNA damage is present (66, 67). As a major effector of mammalian cell progression, many DNA damage checkpoint signaling pathways focus on the inactivation of the CDK/cyclin complex (68, 69). It was reported that CDK12 kinase activity is essential for core DNA replication to gene transcription and G_1–S phase progression (14). Knocking down of CDK12 in human gastric cancer cells induces G_2 phase arrest, demonstrating that CDK12 is involved in checkpoint signaling, which needs more investigation in the detailed molecular basis (14).

CDK12 Regulates MYC Expression and Noncanonical NF-κB Pathway

MYC is an overall transcription factor and core driving force in tumor progression. Inhibition of CDK12 triggers extensive downregulation of c-MYC and its related genes (70–72). Furthermore, MYC downregulation causes replicative stress via accelerating replication rate. The overlapped functions between MYC and CDK12 suggest that targeting CDK12 may be an effective strategy against MYC-dependent human cancers (73–75). It is especially important because MYC has been proved difficult to inhibit directly. NF-κB transcription factors are pivotal for multitudinous cellular processes, including cell proliferation, differentiation, survival, apoptosis, angiogenesis, inflammation, immunity, tumorigenesis, as well as regulating innate and adaptive (76–78). The noncanonical NF-κB (n-NF-κB) pathway is a promising therapeutic strategy for a number of diseases and cancers (79, 80). Canonical NF-κB complexes are retained by IκBα in the cytoplasm; however, n-NF-κB complexes are reserved by p100 (encoded by NFKB2), and the accumulation of NIK (NF-κB–inducing kinase, encoded by MAP3K14). CDK12 was found to promote the ligand-induced increase in MAP3K14 and NFKB2 mRNAs abundances (**Fig. 1C**; ref. 24). This molecular basis suggests that targeting CDK12 to regulate NF-κB may be useful for inhibiting NF-κB–dependent cancers.

CDK12 Activates ErbB–PI3K–AKT, WNT/β-Catenin, and MAPK Signaling

Cell signal transduction is a fundamental and pivotal process in tumor development and progression; dysregulation of various cell signaling pathways promotes cancer cell proliferation, differentiation, motility, metabolic disturbance, and immune response regulation, leading to aberrant tumor growth, invasion, angiogenesis, and metastasis (81). ErbB–PI3K–AKT, WNT/β-catenin, and MAPK signaling are the canonical and critical signal transduction pathways in carcinogenesis. Inhibition of these pathways is one of the main strategies for cancer targeted therapy (82–84). CDK12 can activate ErbB–PI3K–AKT or WNT/β-catenin signaling by phosphorylating RNA POL II to promote tumor initiation, invasion, and metastasis, as well as maintain cancer stem cells self-renewal in breast cancer (**Fig. 1C**; refs. 22, 23). These findings suggest that CDK12 is a novel therapeutic strategy for anticancer therapy, especially for HER2-positive breast cancer. Although the CTD substrates of CDK12 have identified a lot, the non-CTD substrates of CDK12 remain deserted. Our group identified that PAK2 can act as a non-CTD substrate of CDK12. CDK12 phosphorylates PAK2 at threonine 134/169 activating MAPK signaling pathway to promote tumor development in human gastric cancer (**Fig. 1C**; ref. 26). It reminds us CDK12 can act as a novel therapeutic target for human gastric cancer besides VEGFR-2, HER2, and PD-1 (85–88). We believe that other non-CTD substrates and CDK12-related proteins will be discovered and their roles in tumorigenesis will be verified.

Inhibitors of CDK12

Given the critical roles of CDK12 in diseases and cancers, we expect that the inhibitor of CDK12 can be used for cancer prevention and therapy. The overview of CDK inhibitors is shown in Supplementary Table S2. The specific CDK12 inhibitor is CDK12-IN-3, and the others are nonspecific CDK12 inhibitors. Most of them inhibit tumor growth by suppressing DDR, phospho-RNA POLII and have synergistic effect with PARPi.

CDK12 Inhibitors Can Be Used for Targeted Therapy, Can Overcome Drug Resistance, and Can Show Synergistic Effect with Other Anticancer Drugs

It is reported that dinaciclib (also known as SCH 727965), an inhibitor of CDK1, 2, 5, 9, additionally has activity against CDK12 ablating restored HR and reverses PARP inhibitors (PARPi) resistance, supporting the widespread use of combined CDK12 and PARPi in triple-negative breast cancer (TNBC; refs. 89–92). A study verified that inhibiting CDK12 by using THZ1 and THZ531 (covalent small molecule) can suppress expression of DDR genes and is synergistic with PARPi in Ewing sarcoma (33). THZ531 induced significant DDR in hepatocellular carcinoma (HCC) cell lines, and combination of THZ531 with sorafenib showed strong synergy in HCC, suggesting a potential combination therapy strategy for HCC (32). Another study identified that inhibiting CDK7, CDK12, and CDK13 by THZ1 significantly downregulates expression of MYC, leading to dramatic inhibition of tumor

(*Continued.*) The main function of CDK12 is regulating DNA replication, transcription, and mRNA splicing. By participating MYC, NF-κB, DNA damage repair, checkpoint, ErbB–PI3K–AKT, WNT/β-catenin, and MAPK cell signal transduction regulate cancer cell proliferation, growth, survival, and tumor progression. Thus, CDK12 is a promising therapeutic target for cancers. The inhibitors of CDK12 used as anticancer drugs have more possibilities including synergistic effect, therapeutic resistance, immunotherapy, and beyond cancer except targeted therapy. Although function, mechanism, and inhibitors have developed greatly among the past decade, there are still many scientific questions and translation of basic science to the clinical setting to be uncovered. A worthy investigating question is, Will CDK12 be the central hub of oncology?

Table 1. Key distinctions between human CDK subfamilies.

Name	Subcellular location	Molecular weight (kDa)	Cyclin partner	Chromosomal location	Molecular function	Key biological process	Knock out mouse model phenotype	Pertinent to cancer	Reference
CDK1	N, Cys, Cyp, Mic	34.095	CycA, CycB	10q21.2	Host cell receptor for virus entry, kinase, receptor, serine/threonine-protein kinase, transferase	Apoptosis, biological rhythms, cell cycle, cell division, host–virus interaction, mitosis	Embryonic lethality, cellular	RC, LVC, PC, LUC, CC	(111)
CDK2	Ens, N, Cys, Cyp	33.930	CycA, CycE	12q13.2	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division, DNA damage, DNA repair, meiosis	Main abnormal in reproductive system	RC, LVC, HNC	(112)
CDK3	N	35.046	CycE	17q25.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division, mitosis	No abnormal phenotype	/	(113)
CDK4	N, Cyp	33.730	CycD	12q14.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division	Main abnormal in homeostasis/ metabolism	LVC, RC	(114)
CDK5	N,PM	33.304	CycE, Cyc5R	7q36.1	Kinase, serine/threonine-protein kinase, transferase	Apoptosis, biological rhythms, cell cycle, cell division, neurogenesis	Main abnormal in nervous system	/	(115)
CDK6	N, Cyp, Cys	36.938	CycD	7q21.2	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division, differentiation	Main abnormal in immune system	PC, UC	(114)
CDK7	N, Cyp	39.038	CycH	5q13.2	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division, DNA damage, DNA repair, transcription, transcription regulation	Embryonic lethality, integument	RC, PC	(116)
CDK8	N	53.284	CycC	13q12.13	Activator, kinase, repressor, serine/ threonine-protein kinase, transferase	Transcription, transcription regulation	Embryonic lethality, embryo	GC	(117)
CDK9	N, Cyp	42.778	CycT	9q34.11	Kinase, serine/threonine-protein kinase, transferase	DNA damage, DNA repair, transcription, transcription regulation	Embryonic lethality, mortality/aging	RC	(118)
CDK10	Cts	41.038	CycM	16q24.3	Kinase, serine/threonine-protein kinase, transferase	Cillium biogenesis/degradation	Perinatal lethality, renal/ urinary system	PC, UC	(119)
CDK11	N	41.358	CycL	1p36.33	Kinase, serine/threonine-protein kinase, transferase	Apoptosis, cell cycle	Embryonic lethality, integument	RC, MN	(120)
CDK12	N, Cyp	164.155	CycK	17q12	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, mRNA processing, mRNA splicing, transcription, transcription regulation	Embryonic lethality, mortality/aging	BC, GC	(4)
CDK13	N	164.923	CycK	7q14.1	Kinase, serine/threonine-protein kinase, transferase	Host–virus interaction, mRNA processing, mRNA splicing	Preweaning lethality, vision/eye	/	(4)
CDK14	N,PM, Cyp	53.057	CycY	7q21.13	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division, Wnt signaling pathway	Main abnormal in homeostasis/ metabolism	BC, GC	(121)
CDK15	N, Cts, Cyp	49.023	CycY	2q33.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle		/	(1)
CDK16	PM, Cyp	55.716	CycY	Xp11.3	Kinase, serine/threonine-protein kinase, transferase	Differentiation, spermatogenesis	Main abnormal in reproductive system and cellular	LVC	(1)
CDK17	N, Cyp	59.582	CycY	12q23.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle	Preweaning lethality, behavior/neurological	RC	(1)
CDK18	N, Cyp	54.424	CycB	1q32.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle	Main abnormal in behavior/neurological	RC, UC	(122)
CDK19	Cts, N	56.802	CycC	6q21	Kinase, serine/threonine-protein kinase, transferase	Transcription	Main abnormal in behavior/neurological	RC, LVC	(123)
CDK20	N, Cyp	38.695	CycH	9q22.1	Kinase, serine/threonine-protein kinase, transferase	Cell cycle, cell division	Main abnormal in embryo and nervous system	RC, EC	(1)

Abbreviations: BC, breast cancer; CC, cervical cancer; Cts, cytosol; Cyp, cytoplasm; Cys, cytoskeleton; DDC, DNA damage chemotherapy; EC, endometrial cancer; Ens, endosome; GC, gastric cancer; HNC, head and neck cancer; IPA, intronic polyadenylation; LUC, lung cancer; LVC, liver cancer; Mic, mitochondrion; MN, melanoma; N, nucleus; PARPc, PARP cleavage; PARPi, PARP inhibitors; PC, pancreatic cancer; PM, plasma membrane; RC, renal cancer; SEATF, SE-associated transcription factors; UC, urothelial cancer.

growth and MYC expression, providing an effective way for MYC-dependent cancer treatment (75). CDK12 inhibition by THZ1 also resulted in global transcriptional changes and accumulation of NIK via reducing RNA Pol II phosphorylation, which can be used as a therapeutic strategy for autoimmunity and cancers (24). E9 (structurally like dinaciclib) is an irreversible inhibitor of CDK12, leading to a phosphorylated and total RNA poly II decrease in together with MYC and Mcl-1 downregulated, while PARP cleavage increasing (93). Highly selective CDK12 inhibitor CDK12-IN-3 shows potent inhibition of phosphorylation of Ser2 RNA Pol II on the CTD repeat domain in OV90 cells (30). SR-4835, a highly selective dual inhibitor of CDK12 and CDK13, was reported to inhibit TNBC cells by triggering the cleavage of intron polyadenylation sites; the expression of core DNA damage reactive proteins is inhibited, thus promoting the synergistic effect with DNA damage chemotherapy and PARPi (94). One study reported that silencing BRCA1 or CDK12 sensitizes tumor cells to CHK1 inhibitors regardless of p53 status, suggesting that inhibition of CHK1 is a strategy against BRCA1- or CDK12-deficient tumors (34). Our group discovered that procaterol, a clinical used β_2 receptor agonist, can act as CDK12 inhibitor (26, 95, 96). Procaterol inhibits human gastric cancer cell proliferation and tumor growth by inhibiting CDK12 kinase activity, which may be translated into clinic after conducting clinical trial (26). In summary, CDK12 inhibitors can be used for targeted therapy, can overcome drug resistance, and show synergistic effect with other anticancer drugs in different human cancers. But there is no CDK12 inhibitor now used in clinic for CDK12-targeted therapy.

New Combination Therapy: CDK12 Inhibitor with Immune Reagents

The new era of immunotherapy has changed the practice of clinical oncology. There is an urgent need to develop new strategies to modalize the clinical outcomes of immunotherapy and to extend its benefits beyond the PD-1/PD-L1 signaling pathway to a broader population of patients with cancer (97). Several nonclassical molecular immune targets have been shown to act as feedback resistance circuits to shut down the classical immune checkpoint inhibitor–mediated antitumor immune response, including CD40, CD47, CD134, T-cell inducible costimulator, Toll-like receptors, and CDK12 (27, 98–108). Novel combinatorial approaches to improve the effect of cancer immunotherapy are needed based on the classical immunotherapies and strategies. It was reported that deletions of CDK12 bialleles showed genomic instability and increased neoantigen load, followed by enhanced tumor T-cell infiltration, and 50% of patients with mCRPC responded positively to PD-1 blocking (reduced PSA levels; refs. 27, 109). This report suggests that CDK12 loss in mCRPC may act as a hopeful prognostic biomarker for the potential benefits of immune checkpoint immunotherapy, and a new combination method applying CDK12 inhibitors as potential sensitizing agents to heighten the response to immune checkpoint antibody therapy may be useful in prostate tumors. We expect that the combination of CDK12 inhibitors with immune therapy has a wider application for the foreseeable future. In addition, it was reported that a novel compound (DDD853651/GSK3186899) is efficacious in a *Visceral leishmaniasis* (VL) mouse model by inhibiting CDC2-related kinase 12 (CRK12), the *Leishmania* homolog of CDK12, indicating that targeting CDK12 may have broad indications in human diseases beyond cancer (110).

Conclusions

CDK12 is emerging as a critical signaling molecular for cancer development. As a transcription associated CDK, CDK12 binds with cyclin K phosphorylate RNA Pol II at CTD promoting transcription elongation. CDK12 can also interact with RNA processing factors to regulate RNA splicing. Furthermore, CDK12 mediated phosphorylation of RNA Pol II couples mRNA 3′ end processing to participate in transcription termination. Besides, CDK12 plays critical role in regulating intronic polyadenylation, epigenetics, as well as translation. CDK12 alterations were found in different human cancers. Loss of function of CDK12 mutations or deletion can cause genomic instability, which may act as a biomarker in ovarian cancer and prostate cancer. The critical role of CDK12 in tumorigenesis and progress may mainly via activating c-MYC/β-catenin signaling, ErbB–PI3K–AKT, or WNT signaling cascades, MAPK signaling, noncanonical NF-κB pathway, and DDR signaling, and a number of studies have proved that CDK12 inhibitor is highly effective in cancer-targeted therapy and combination therapy or together with immune therapy (**Fig. 1D**).

Although CDK12 has received sustained attention for nearly 15 years, there are still many key questions waiting for investigation. For example, how CDK12 interacts with others to regulate complex molecular processes? What intrinsic redundancies are in place? How CDK12 works with other CDK members to affect cancer hallmarks? What are the consequences of CDK12 loss or accumulation in tumorigenesis of different cancer types and the posttranslational modification including phosphorylation, acetylation, methylation, ubiquitination, and degradation? Furthermore, the diseases beyond cancer that may be affected by CDK12 and benefited from CDK12 inhibitors also remain to be studied. In addition, much more work on developing effective specific CDK12 inhibitors is important, as the current inhibitors of CDK12 have not been used clinically yet. To uncover the answers of these questions, researchers may need more work, such as using CDK12 conditional knockout mouse to verify the role of CDK12 in tumorigenesis precisely for different cancer types. Also, we need to generate the phospho-CDK12 antibody to elucidate the consequence of phosphorylated CDK12 in diseases and cancers. As for CDK12 inhibitor, researchers can also find some natural compounds from herbs or fruits, which may inhibit CDK12 and used for chemoprevention or therapy of CDK12-related cancers.

Understanding the function, mechanism, and inhibition of CDK12 is an exciting area of oncology. We are waiting for the entry of CDK12 inhibitors into clinical trials, as well as looking forward to the identification of an effective combination therapy of CDK12 inhibitors with other anticancer agents or immune checkpoint inhibitors with elucidative meticulous mechanisms.

Authors' Disclosures

No disclosures were reported..

Acknowledgments

This work was supported by the China-US (Henan) Hormel Cancer Institute.

Received July 8, 2020; revised August 23, 2020; accepted September 16, 2020; published first September 21, 2020.

References

1. Malumbres M. Cyclin-dependent kinases. Genome Biol 2014;15:122.
2. Chou J, Quigley DA, Robinson TM, Feng FY, Ashworth A. Transcription-associated cyclin-dependent kinases as targets and biomarkers for cancer therapy. Cancer Discov 2020;10:351–70.
3. Dar AA, Nosrati M, Bezrookove V, de Semir D, Majid S, Thummala S, et al. The role of BPTF in melanoma progression and in response to BRAF-targeted therapy. J Natl Cancer Inst 2015;107:9.
4. Greenleaf AL. Human CDK12 and CDK13, multi-tasking CTD kinases for the new millenium. Transcription 2018;10:91–110.
5. Ko TK, Kelly E, Pines J. CrkRS: a novel conserved Cdc2-related protein kinase that colocalises with SC35 speckles. J Cell Sci 2001;114:2591–603.
6. Iorns E, Martens-de Kemp SR, Lord CJ, Ashworth A. CRK7 modifies the MAPK pathway and influences the response to endocrine therapy. Carcinogenesis 2009;30:1696–701.
7. Juan HC, Lin Y, Chen HR, Fann MJ. Cdk12 is essential for embryonic development and the maintenance of genomic stability. Cell Death Differ 2016;23:1038–48.
8. Chen HR, Lin GT, Huang CK, Fann MJ. Cdk12 and Cdk13 regulate axonal elongation through a common signaling pathway that modulates Cdk5 expression. Exp Neurol 2014;261:10–21.
9. Chen HH, Wang YC, Fann MJ. Identification and characterization of the CDK12/cyclin L1 complex involved in alternative splicing regulation. Mol Cell Biol 2006;26:2736–45.
10. Bartkowiak B, Greenleaf AL. Expression, purification, and identification of associated proteins of the full-length hCDK12/CyclinK complex. J Bio Chem 2015;290:1786–95.
11. Blazek D, Kohoutek J, Bartholomeeusen K, Johansen E, Hulinkova P, Luo Z, et al. The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. Genes Dev 2011;25:2158–72.
12. Cheng SW, Kuzyk MA, Moradian A, Ichu TA, Chang VC, Tien JF, et al. Interaction of cyclin-dependent kinase 12/CrkRS with cyclin K1 is required for the phosphorylation of the C-terminal domain of RNA polymerase II. Mol Cell Biol 2012;32:4691–704.
13. Tien JF, Mazloomian A, Cheng SWG, Hughes CS, Chow CCT, Canapi LT, et al. CDK12 regulates alternative last exon mRNA splicing and promotes breast cancer cell invasion. Nucleic Acids Res 2017;45:6698–716.
14. Chirackal Manavalan AP, Pilarova K, Kluge M, Bartholomeeusen K, Rajecky M, Oppelt J, et al. CDK12 controls G1/S progression by regulating RNAPII processivity at core DNA replication genes. EMBO Rep 2019;20:e47592.
15. Dubbury SJ, Boutz PL, Sharp PA. CDK12 regulates DNA repair genes by suppressing intronic polyadenylation. Nature 2018;564:141–5.
16. Krajewska M, Dries R, Grassetti AV, Dust S, Gao Y, Huang H, et al. CDK12 loss in cancer cells affects DNA damage response genes through premature cleavage and polyadenylation. Nat Commun 2019;10:1757.
17. Choi SH, Martinez TF, Kim S, Donaldson C, Shokhirev MN, Saghatelian A, et al. CDK12 phosphorylates 4E-BP1 to enable mTORC1-dependent translation and mitotic genome stability. Genes Dev 2019;33:418–35.
18. Popova T, Manie E, Boeva V, Battistella A, Goundiam O, Smith NK, et al. Ovarian cancers harboring inactivating mutations in CDK12 display a distinct genomic instability pattern characterized by large tandem duplications. Cancer Res 2016;76:1882–91.
19. Zhang X, Nguyen KD, Rudnick PA, Roper N, Kawaler E, Maity TK, et al. Quantitative mass spectrometry to interrogate proteomic heterogeneity in metastatic lung adenocarcinoma and validate a novel somatic mutation CDK12-G879V. Mol Cell Proteomics 2019;18:622–41.
20. Sokol ES, Pavlick D, Frampton GM, Ross JS, Miller VA, Ali SM, et al. Pan-cancer analysis of CDK12 loss-of-function alterations and their association with the focal tandem-duplicator phenotype. Oncologist 2019;24:1526–33.
21. Nguyen B, Mota JM, Nandakumar S, Stopsack KH, Weg E, Rathkopf D, et al. Pan-cancer analysis of CDK12 alterations identifies a subset of prostate cancers with distinct genomic and clinical characteristics. Eur Urol 2020;78:671–9.
22. Peng F, Yang C, Kong Y, Huang X, Chen Y, Zhou Y, et al. CDK12 promotes breast cancer progression and maintains stemness by activating c-myc/beta -catenin signaling. Curr Cancer Drug Tar 2020;20:156–65.
23. Choi HJ, Jin S, Cho H, Won HY, An HW, Jeong GY, et al. CDK12 drives breast tumor initiation and trastuzumab resistance via WNT and IRS1-ErbB-PI3K signaling. EMBO Rep 2019;20:e48058.
24. Henry KL, Kellner D, Bajrami B, Anderson JE, Beyna M, Bhisetti G, et al. CDK12-mediated transcriptional regulation of noncanonical NF-κB components is essential for signaling. Sci Signal 2018;11:eaam8216.
25. Naidoo K, Wai PT, Maguire SL, Daley F, Haider S, Kriplani D, et al. Evaluation of CDK12 protein expression as a potential novel biomarker for DNA damage response-targeted therapies in breast cancer. Mol Cancer Ther 2018;17:306–15.
26. Liu H, Shin SH, Chen H, Liu T, Li Z, Hu Y, et al. CDK12 and PAK2 as novel therapeutic targets for human gastric cancer. Theranostics 2020;10:6201–15.
27. Wu YM, Cieslik M, Lonigro RJ, Vats P, Reimers MA, Cao X, et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. Cell 2018;173:1770–82.
28. Marshall CH, Imada EL, Tang Z, Marchionni L, Antonarakis ES. CDK12 inactivation across solid tumors: an actionable genetic subtype. Oncoscience 2019;6:312–6.
29. Choi SH, Kim S, Jones KA. Gene expression regulation by CDK12: a versatile kinase in cancer with functions beyond CTD phosphorylation. Exp Mol Med 2020;52:762–71.
30. Johannes JW, Denz CR, Su N, Wu A, Impastato AC, Mlynarski S, et al. Structure-based design of selective noncovalent CDK12 inhibitors. ChemMedChem 2018;13:231–5.
31. CDK12/13 inhibitor SR-4835 is active in triple-negative breast cancer. Cancer Discov 2019;9:1644.
32. Wang C, Wang H, Lieftink C, du Chatinier A, Gao D, Jin G, et al. CDK12 inhibition mediates DNA damage and is synergistic with sorafenib treatment in hepatocellular carcinoma. Gut 2020;69:727–36.
33. Iniguez AB, Stolte B, Wang EJ, Conway AS, Alexe G, Dharia NV, et al. EWS/FLI confers tumor cell synthetic lethality to CDK12 inhibition in Ewing sarcoma. Cancer Cell 2018;33:202–16.
34. Paculová H, Kramara J, S?imečková S?, Fedr R, Souček K, Hylse O, et al. BRCA1 or CDK12 loss sensitizes cells to CHK1 inhibitors. Tumour Biol 2017;39:1010428317727479.
35. Lei T, Zhang P, Zhang X, Xiao X, Zhang J, Qiu T, et al. Cyclin K regulates prereplicative complex assembly to promote mammalian cell proliferation. Nat Commun 2018;9:1876.
36. Liang K, Gao X, Gilmore JM, Florens L, Washburn MP, Smith E, et al. Characterization of human cyclin-dependent kinase 12 (CDK12) and CDK13 complexes in C-terminal domain phosphorylation, gene transcription, and RNA processing. Mol Cell Biol 2015;35:928–38.
37. Ahn SH, Kim M, Buratowski S. Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3′ end processing. Mol Cell 2004;13:67–76.
38. Davidson L, Muniz L, West S. 3′ end formation of pre-mRNA and phosphorylation of Ser2 on the RNA polymerase II CTD are reciprocally coupled in human cells. Genes Dev 2014;28:342–56.
39. Li X, Chatterjee N, Spirohn K, Boutros M, Bohmann D. Cdk12 is a gene-selective RNA polymerase II kinase that regulates a subset of the transcriptome, including Nrf2 target genes. Sci Rep 2016;6:21455.
40. Bartkowiak B, Liu P, Phatnani HP, Fuda NJ, Cooper JJ, Price DH, et al. CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1. Genes Dev 2010;24:2303–16.
41. Zhang K, Zhang M, Zhu J, Hong W. Screening of gene mutations associated with bone metastasis in nonsmall cell lung cancer. J Cancer Res Ther 2016;12:C186–90.
42. Biswas R, Gao S, Cultraro CM, Maity TK, Venugopalan A, Abdullaev Z, et al. Genomic profiling of multiple sequentially acquired tumor metastatic sites from an "exceptional responder" lung adenocarcinoma patient reveals extensive genomic heterogeneity and novel somatic variants driving treatment response. Cold Spring Harb Mol Case Stud 2016;2:a001263.
43. Chen B, Zhang G, Wei G, Wang Y, Guo L, Lin J, et al. Heterogeneity of genomic profile in patients with HER2-positive breast cancer. Endocr Relat Cancer 2020;27:153–62.
44. Luoh SW. Amplification and expression of genes from the 17q11 approximately q12 amplicon in breast cancer cells. Cancer Genet Cytogen 2002;136:43–7.
45. Sircoulomb F, Bekhouche I, Finetti P, Adelaide J, Ben Hamida A, Bonansea J, et al. Genome profiling of ERBB2-amplified breast cancers. BMC Cancer 2010;10:539.
46. Joshi PM, Sutor SL, Huntoon CJ, Karnitz LM. Ovarian cancer-associated mutations disable catalytic activity of CDK12, a kinase that promotes homologous recombination repair and resistance to cisplatin and poly(ADP-ribose) polymerase inhibitors. J Bio Chem 2014;289:9247–53.
47. Ekumi KM, Paculova H, Lenasi T, Pospichalova V, Bosken CA, Rybarikova J, et al. Ovarian carcinoma CDK12 mutations misregulate expression of DNA

repair genes via deficient formation and function of the Cdk12/CycK complex. Nucleic Aacids Res 2015;43:2575–89.
48. Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, Simpson JT, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009;462:1005–10.
49. McBride DJ, Etemadmoghadam D, Cooke SL, Alsop K, George J, Butler A, et al. Tandem duplication of chromosomal segments is common in ovarian and breast cancer genomes. J Pathol 2012;227:446–55.
50. Menghi F, Barthel FP, Yadav V, Tang M, Ji B, Tang Z, et al. The tandem duplicator phenotype is a prevalent genome-wide cancer configuration driven by distinct gene mutations. Cancer Cell 2018;34:197–210.
51. Quigley DA, Dang HX, Zhao SG, Lloyd P, Aggarwal R, Alumkal JJ, et al. Genomic hallmarks and structural variation in metastatic prostate cancer. Cell 2018;174:758–69.
52. van Dessel LF, van Riet J, Smits M, Zhu Y, Hamberg P, van der Heijden MS, et al. The genomic landscape of metastatic castration-resistant prostate cancers reveals multiple distinct genotypes with potential clinical impact. Nat Commun 2019;10:5251.
53. Viswanathan SR, Ha G, Hoff AM, Wala JA, Carrot-Zhang J, Whelan CW, et al. Structural alterations driving castration-resistant prostate cancer revealed by linked-read genome sequencing. Cell 2018;174:433–47.
54. Reimers MA, Yip SM, Zhang L, Cieslik M, Dhawan M, Montgomery B, et al. Clinical outcomes in cyclin-dependent kinase 12 mutant advanced prostate cancer. Euro Urol 2020;77:333–41.
55. Markowski MC, Shenderov E, Eisenberger MA, Kachhap S, Pardoll DM, Denmeade SR, et al. Extreme responses to immune checkpoint blockade following bipolar androgen therapy and enzalutamide in patients with metastatic castration resistant prostate cancer. Prostate 2020;80:407–11.
56. Stopsack KH, Nandakumar S, Wibmer AG, Haywood S, Weg ES, Barnett ES, et al. Oncogenic genomic alterations, clinical phenotypes, and outcomes in metastatic castration-sensitive prostate cancer. Clin Cancer Res 2020;26:3230–8.
57. Abida W, Campbell D, Patnaik A, Shapiro JD, Sautois B, Vogelzang NJ, et al. Non-BRCA DNA damage repair gene alterations and response to the PARP inhibitor rucaparib in metastatic castration-resistant prostate cancer: analysis from the phase II TRITON2 Study. Clin Cancer Res 2020;26:2487–96.
58. Sokolova AO, Yu EY, Cheng HH. Honing in on PARPi response in prostate cancer: from HR pathway to gene-by-gene granularity. Clin Cancer Res 2020;26:2439–40.
59. Lv SD, Wang HY, Yu XP, Zhai QL, Wu YB, Wei Q, et al. Integrative molecular characterization of Chinese prostate cancer specimens. Asian J Androl 2020;22:162–8.
60. Guffanti F, Fruscio R, Rulli E, Damia G. The impact of DNA damage response gene polymorphisms on therapeutic outcomes in late stage ovarian cancer. Sci Rep 2016;6:38142.
61. Mehta A, Haber JE. Sources of DNA double-strand breaks and models of recombinational DNA repair. Cold Spring Harb Perspect Biol 2014;6:a016428.
62. Mitelman F, Johansson B, Mertens F. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 2007;7:233–45.
63. Cannan WJ, Pederson DS. Mechanisms and consequences of double-strand DNA break formation in chromatin. J Cell Physiol 2016;231:3–14.
64. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 2005;5:773–85.
65. Tighe A, Johnson VL, Taylor SS. Truncating APC mutations have dominant effects on proliferation, spindle checkpoint control, survival and chromosome stability. J Cell Sci 2004;117:6339–53.
66. Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science 1989;246:629–34.
67. Nojima H. Cell cycle checkpoints, chromosome stability and the progression of cancer. Hum Cell 1997;10:221–30.
68. Sorensen CS, Syljuasen RG. Safeguarding genome integrity: the checkpoint kinases ATR, CHK1 and WEE1 restrain CDK activity during normal DNA replication. Nucleic Acids Res 2012;40:477–86.
69. Palmer N, Kaldis P. Less-well known functions of cyclin/CDK complexes. Semin Cell Dev Biol 2020;1:9.
70. Schaub FX, Dhankani V, Berger AC, Trivedi M, Richardson AB, Shaw R, et al. Pan-cancer alterations of the MYC oncogene and its proximal network across the Cancer Genome Atlas. Cell Syst 2018;6:282–300.
71. Toyoshima M, Howie HL, Imakura M, Walsh RM, Annis JE, Chang AN, et al. Functional genomics identifies therapeutic targets for MYC-driven cancer. Proc Natl Acad Sci U S A 2012;109:9545–50.
72. Delehouze C, Godl K, Loaec N, Bruyere C, Desban N, Oumata N, et al. CDK/CK1 inhibitors roscovitine and CR8 downregulate amplified MYCN in neuroblastoma cells. Oncogene 2014;33:5675–87.
73. Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, et al. Non-transcriptional control of DNA replication by c-Myc. Nature 2007;448:445–51.
74. Baluapuri A, Wolf E, Eilers M. Target gene-independent functions of MYC oncoproteins. Nat Rev Mol Cell Biol 2020;21:255–67.
75. Zeng M, Kwiatkowski NP, Zhang T, Nabet B, Xu M, Liang Y, et al. Targeting MYC dependency in ovarian cancer through inhibition of CDK7 and CDK12/13. eLife 2018;7:e39030.
76. Ferrandino F, Grazioli P, Bellavia D, Campese AF, Screpanti I, Felli MP. Notch and NF-kappaB: coach and players of regulatory T-cell response in cancer. Front Immunol 2018;9:2165.
77. Taniguchi K, Karin M. NF-kappaB, inflammation, immunity and cancer: coming of age. Nat Rev Immunol 2018;18:309–24.
78. Ahmad A, Ahsan H. Ras-mediated activation of NF-κB and DNA damage response in carcinogenesis. Cancer Invest 2020;38:185–208.
79. Cai Q, Tu M, Xu-Monette ZY, Sun R, Manyam GC, Xu X, et al. NF-kappaB p50 activation associated with immune dysregulation confers poorer survival for diffuse large B-cell lymphoma patients with wild-type p53. Mod Pathol 2017;30:854–76.
80. Cildir G, Low KC, Tergaonkar V. Noncanonical NF-kappaB signaling in health and disease. Trends Mol Med 2016;22:414–29.
81. Ortega MA, Fraile-Martinez O, Asunsolo A, Bujan J, Garcia-Honduvilla N, Coca S. Signal transduction pathways in breast cancer: the important role of PI3K/Akt/mTOR. J Oncol 2020;2020:9258396.
82. Reddy D, Ghosh P, Kumavath R. Strophanthidin attenuates MAPK, PI3K/AKT/mTOR, and Wnt/beta-catenin signaling pathways in human cancers. Front Oncol 2019;9:1469.
83. Reddy D, Kumavath R, Tan TZ, Ampasala DR, Kumar AP. Peruvoside targets apoptosis and autophagy through MAPK Wnt/beta-catenin and PI3K/AKT/mTOR signaling pathways in human cancers. Life Sci 2020;241:117147.
84. Reddy D, Kumavath R, Ghosh P, Barh D. Lanatoside C induces G2/M cell cycle arrest and suppresses cancer cell growth by attenuating MAPK, Wnt, JAK-STAT, and PI3K/AKT/mTOR signaling pathways. Biomolecules 2019;9:792.
85. Van Cutsem E, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. Lancet 2016;388:2654–64.
86. Bonelli P, Borrelli A, Tuccillo FM, Silvestro L, Palaia R, Buonaguro FM. Precision medicine in gastric cancer. World J Gastro Oncol 2019;11:804–29.
87. Lordick F, Shitara K, Janjigian YY. New agents on the horizon in gastric cancer. Ann Oncol 2017;28:1767–75.
88. Kataoka K, Shiraishi Y, Takeda Y, Sakata S, Matsumoto M, Nagano S, et al. Aberrant PD-L1 expression through 3′-UTR disruption in multiple cancers. Nature 2016;534:402–6.
89. Johnson SF, Cruz C, Greifenberg AK, Dust S, Stover DG, Chi D, et al. CDK12 inhibition reverses de novo and acquired PARP inhibitor resistance in BRCA wild-type and mutated models of triple-negative breast cancer. Cell Rep 2016;17:2367–81.
90. Novotná E, Büküm N, Hofman J, Flaxová M, Kouklíková E, Louvarová D, et al. Aldo-keto reductase 1C3 (AKR1C3): a missing piece of the puzzle in the dinaciclib interaction profile. Arch Toxicol 2018;92:2845–57.
91. Washino S, Rider LC, Romero L, Jillson LK, Affandi T, Ohm AM, et al. Loss of MAP3K7 sensitizes prostate cancer cells to CDK1/2 inhibition and DNA damage by disrupting homologous recombination. Mol Cancer Res 2019;17:1985–98.
92. Parry D, Guzi T, Shanahan F, Davis N, Prabhavalkar D, Wiswell D, et al. Dinaciclib (SCH 727965), a novel and potent cyclin-dependent kinase inhibitor. Mol Cancer Ther 2010;9:2344–53.
93. Gao Y, Zhang T, Terai H, Ficarro SB, Kwiatkowski N, Hao MF, et al. Overcoming resistance to the THZ series of covalent transcriptional CDK inhibitors. Cell Chem Biol 2018;25:135–42.
94. Quereda V, Bayle S, Vena F, Frydman SM, Monastyrskyi A, Roush WR, et al. Therapeutic targeting of CDK12/CDK13 in triple-negative breast cancer. Cancer Cell 2019;36:545–58.
95. Kainuma K, Kobayashi T, D'Alessandro-Gabazza CN, Toda M, Yasuma T, Nishihama K, et al. β(2) adrenergic agonist suppresses eosinophil-induced epithelial-to-mesenchymal transition of bronchial epithelial cells. Respir Res 2017;18:79.
96. Agarwal D, Glasel JA. Differential effects of opioid and adrenergic agonists on proliferation in a cultured cell line. Cell Proliferat 1999;32:215–29.

97. Omar HA, Tolba MF. Tackling molecular targets beyond PD-1/PD-L1: novel approaches to boost patients' response to cancer immunotherapy. Crit Rev Oncol Hematol 2019;135:21–9.
98. Eliopoulos AG, Young LS. The role of the CD40 pathway in the pathogenesis and treatment of cancer. Curr Opin Pharmacol 2004;4:360–7.
99. Byrne KT, Vonderheide RH. CD40 stimulation obviates innate sensors and drives T cell immunity in cancer. Cell Rep 2016;15:2719–32.
100. Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD Jr, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell 2009;138:286–99.
101. Edris B, Weiskopf K, Volkmer AK, Volkmer JP, Willingham SB, Contreras-Trujillo H, et al. Antibody therapy targeting the CD47 protein is effective in a model of aggressive metastatic leiomyosarcoma. P Natl Acad Sci of U S A 2012; 109:6656–61.
102. Weiskopf K, Jahchan NS, Schnorr PJ, Cristea S, Ring AM, Maute RL, et al. CD47-blocking immunotherapies stimulate macrophage-mediated destruction of small-cell lung cancer. J Clin Invest 2016;126:2610–20.
103. Weinberg AD, Morris NP, Kovacsovics-Bankowski M, Urba WJ, Curti BD. Science gone translational: the OX40 agonist story. Immunol Rev 2011;244: 218–31.
104. Fu T, He Q, Sharma P. The ICOS/ICOSL pathway is required for optimal antitumor responses mediated by anti-CTLA-4 therapy. Cancer Res 2011;71: 5445–54.
105. Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, Vence LM, et al. VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med 2017;23:551–5.
106. DuPage M, Chopra G, Quiros J, Rosenthal WL, Morar MM, Holohan D, et al. The chromatin-modifying enzyme Ezh2 is critical for the maintenance of regulatory T cell identity after activation. Immunity 2015;42: 227–38.
107. Goswami S, Apostolou I, Zhang J, Skepner J, Anandhan S, Zhang X, et al. Modulation of EZH2 expression in T cells improves efficacy of anti-CTLA-4 therapy. J Clin Invest 2018;128:3813–8.
108. Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol 2007;7:179–90.
109. Antonarakis ES. Cyclin-dependent kinase 12, immunity, and prostate cancer. N Engl J Med 2018;379:1087–9.
110. Wyllie S, Thomas M, Patterson S, Crouch S, De Rycker M, Lowe R, et al. Cyclin-dependent kinase 12 is a drug target for visceral leishmaniasis. Nature 2018;560: 192–7.
111. Ravindran Menon D, Luo Y, Arcaroli JJ, Liu S, KrishnanKutty LN, Osborne DG, et al. CDK1 interacts with Sox2 and promotes tumor initiation in human melanoma. Cancer Res 2018;78:6561–74.
112. Gopinathan L, Tan SL, Padmakumar VC, Coppola V, Tessarollo L, Kaldis P. Loss of Cdk2 and cyclin A2 impairs cell proliferation and tumorigenesis. Cancer Res 2014;74:3870–9.
113. Braun K, Hölzl G, Soucek T, Geisen C, Möröy T, Hengstschläger M. Investigation of the cell cycle regulation of cdk3-associated kinase activity and the role of cdk3 in proliferation and transformation. Oncogene 1998;17:2259–69.
114. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: from discovery to therapy. Cancer Discov 2016;6:353–67.
115. Pozo K, Bibb JA. The emerging role of Cdk5 in cancer. Trends Cancer 2016;2: 606–18.
116. Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, et al. Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. Nature 2014;511:616–20.
117. Roninson IB, Győrffy B, Mack ZT, Shtil AA, Shtutman MS, Chen M, et al. Identifying cancers impacted by CDK8/19. Cells 2019;8:821.
118. Franco LC, Morales F, Boffo S, Giordano A. CDK9: a key player in cancer and other diseases. J Cell Biochem 2018;119:1273–84.
119. Iorns E, Turner NC, Elliott R, Syed N, Garrone O, Gasco M, et al. Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. Cancer Cell 2008;13:91–104.
120. Dos Santos Paparidis NF, Canduri F. The emerging picture of CDK11: genetic, functional and medicinal aspects. Curr Med Chem 2018;25:880–8.
121. Ou-Yang J, Huang LH, Sun XX. Cyclin-dependent kinase 14 promotes cell proliferation, migration and invasion in ovarian cancer by inhibiting Wnt signaling pathway. Gynecol Obstet Inves 2017;82:230–9.
122. Barone G, Staples CJ, Ganesh A, Patterson KW, Bryne DP, Myers KN, et al. Human CDK18 promotes replication stress signaling and genome stability. Nucleic Acids Res 2016;44:8772–85.
123. Sánchez-Martínez C, Lallena MJ, Sanfeliciano SG, de Dios A. Cyclin dependent kinase (CDK) inhibitors as anticancer drugs: Recent advances (2015–2019). Bioorg Med Chem Lett 2019;29:126637.